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Writing DNA with GenoCAD^{TM}

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ABSTRACT

Chemical synthesis of custom DNA made to order calls for software streamlining the design of synthetic DNA sequences. GenoCAD^{TM} (www.genocad.org) is a free web-based application to design protein expression vectors, artificial gene networks and other genetic constructs composed of multiple functional blocks called genetic parts. By capturing design strategies in grammatical models of DNA sequences, GenoCAD guides the user through the design process. By successively clicking on icons representing structural features or actual genetic parts, complex constructs composed of dozens of functional blocks can be designed in a matter of minutes. GenoCAD automatically derives the construct sequence from its comprehensive libraries of genetic parts. Upon completion of the design process, users can download the sequence for synthesis or further analysis. Users who elect to create a personal account on the system can customize their workspace by creating their own parts libraries, adding new parts to the libraries, or reusing designs to quickly generate sets of related constructs.

INTRODUCTION

In order to fully reap the potential benefits of *de novo* chemical gene synthesis (1) it has become necessary to develop tools and methodologies to streamline the design of custom DNA sequences (2). Protein expression for structural studies (3), functional genomics (4,5), metabolic engineering (6,7), or gene expression studies (8–11) are only some of the numerous possible applications of this emerging technology. Beyond small scale genetic constructs encompassing no more than a few interacting genes, it becomes possible to reengineer viral (12–15), bacterial (16), and even eukaryotic (17) genomes. While the number of users of this technology increases, so does the need to streamline the design of synthetic DNA sequences. GenoCAD is a web-based application filling this need by providing users with an integrated graphical development environment that no other software provides.

GenoCAD's design philosophy derives from the notion of genetic parts, which was first articulated to analyze genomics data (18). Thinking of genetic systems as composed of parts, each with its own function and characteristics, is akin to the way parts are described and used in various engineering fields. Designing complex systems through a bottom up integration of components is a dominant paradigm in engineering. It was therefore natural that engineers approaching DNA as an engineering substrate, rather than a natural macromolecule, used the notion of biological parts as building blocks (19,20). For instance, promoters, ribosome-binding sites (RBS), genes and terminators are all categories of parts that are needed for designing complex prokaryotic genetic constructs such as switches (21–23) and oscillators (23–25). One could argue that systematic efforts to decompose biological sequences into functional modules that can be recombined to meet user-defined specifications is one of the most distinctive features of synthetic biology compared to more traditional uses of recombinant DNA technologies (2,26–28).

GenoCAD facilitates the design of artificial DNA sequences in three ways. First, GenoCAD includes a flexible system to manage libraries of public and user-defined genetic parts. Second, GenoCAD relies on formal design strategies to guide both novice and experienced users in the design of structurally valid constructs for various biological applications. Finally, GenoCAD's sophisticated data model enables individual users and research groups to customize their workspace to their specific needs.

FLEXIBLE MANAGEMENT OF GENETIC PARTS LIBRARIES

Nothing better attests the benefits of a parts-based approach to the design of genetic constructs than the success of the Registry of Standard Biological Parts (www.partsregistry.org). By defining the BioBrick^{TM} standard allowing the composition of parts and implementing mechanisms to share parts, the Registry has been critical in fostering the development of a vibrant synthetic biology community (1,19,20,29). We recently analyzed the content of the Registry database and the associated collection of clones to better understand how the successes and limitations of this pioneering experiment could guide the

*To whom correspondence should be addressed. Tel: +540 231 0403; Fax: +540 231 2606; Email: peccoud@vt.edu development of a second generation of registries of biological parts (29). GenoCAD attempts to refine some of the concepts upon which the Registry was developed.

By assuming that genetic designs can be synthesized, GenoCAD eliminates the need for standardizing the means by which parts are connected. It also eliminates the need to develop a collection of bacterial clones to manage the physical implementation of the parts. Our analysis also stressed the importance of basic parts used to generate new combinations of parts with specific functions. Ensuring the accuracy of the sequence and annotation of the basic parts is essential since inaccuracies at this level may affect numerous designs. As a result of this observation, GenoCAD parts libraries are exclusively composed of basic parts while sequences composed of multiple parts are called designs. The libraries of parts available to all GenoCAD users are limited to sequences used in peer-reviewed publications or commercial vectors. Parts are curated by a small number of experts according to a process that will be described in a future publication.

Categorizing parts into functional groups has also proved challenging as the number and diversity of parts increases. It would be, for instance, questionable to record bacterial and eukaryotic promoters in the same group. Developing a more granular categorization system may lead to an exponential growth of categories that would prove cumbersome to navigate. GenoCAD overcomes this challenge by relying on the notion of grammar (30). A grammar is composed of rules describing the structure of DNA sequences. One of the rules of the *Escherichia coli* Expression Grammar is CAS→PRO CIS TER which reads: an expression cassette is composed of a promoter, a cistron and a terminator. Another rule of the same grammar is CIS→RBS GEN (a cistron is composed of a RBS and a gene). The two rules can be used successively to create a basic expression cassette PRO RBS GEN TER. Different grammars can be developed for different applications and each grammar has its own parts categorization hierarchy. This approach ensures, for instance, that parts suitable for designing constructs for specific organisms like *E. coli* or yeast can easily be identified. It also enables the development of grammars for specific applications like protein production, homologous recombination in yeast, etc. Instead of attempting to develop a universal parts categorization system, GenoCAD provides a generic framework for the development of smaller more manageable application-specific parts-libraries. The 'Parts' tab of the GenoCAD user interface provides a parts library browser (Figure 1).

POINT-AND-CLICK DESIGN OF GENETIC CONSTRUCTS

In addition to providing a hierarchy of categories, grammars include sets of rewriting rules that formalize design strategies for various types of genetic constructs (2,30). The design feature of GenoCAD embeds the grammars in a graphical user interface that leads users through the design workflow formalized in the grammar. Grammars usually prompt users to begin by choosing high-level structures of their system and systematically decomposing them into individual part categories. The last step of the design process consists in selecting actual parts corresponding to specific DNA sequences (Figure 2). When starting from one of the public design templates, users can quickly design constructs by simply selecting parts in a parts library instead of going through the entire design process described below.

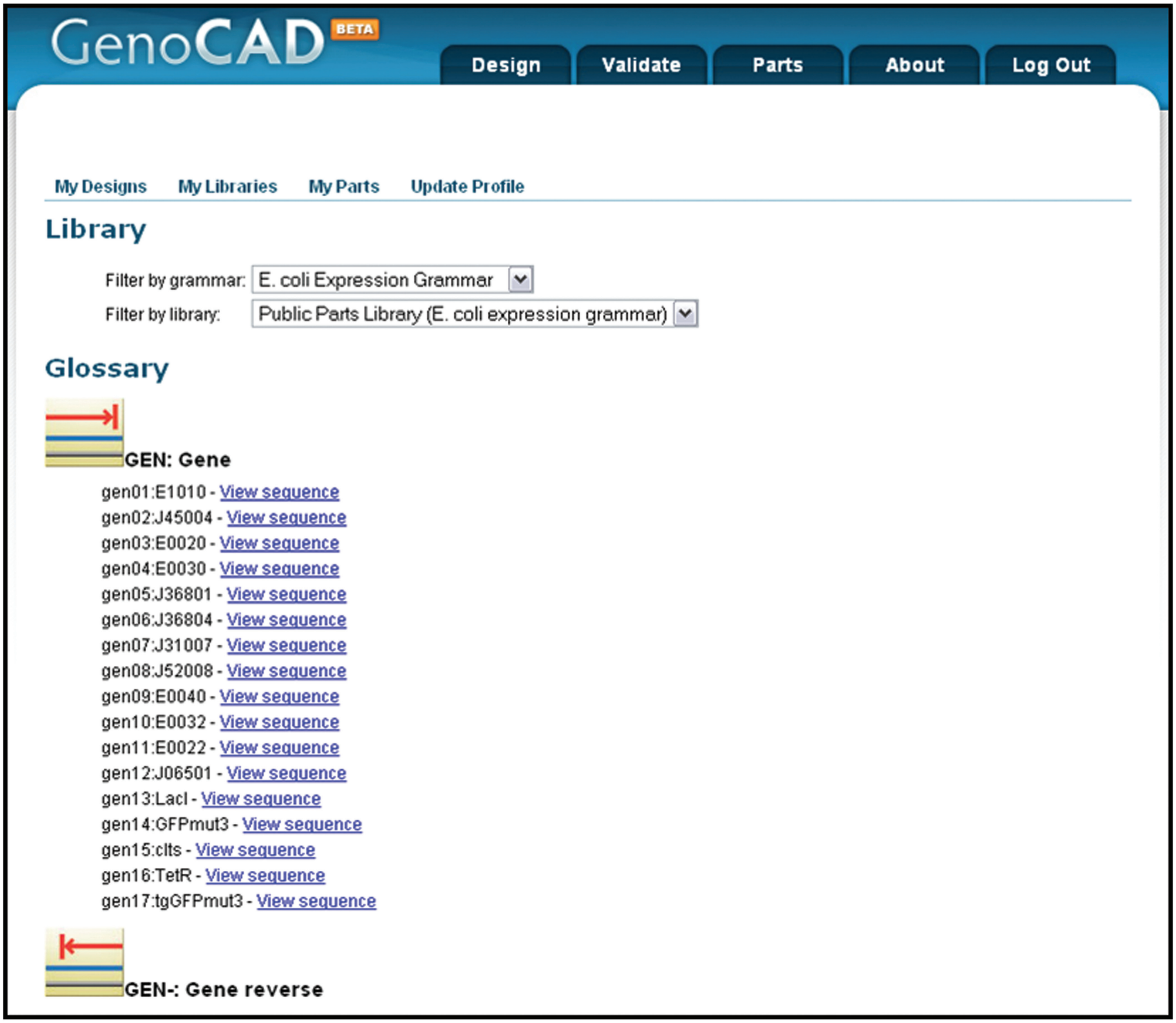
Here, we use the design of a bistable genetic switch to illustrate GenoCAD's workflow (22). Selection of a grammar and an associated parts library (Figure 2) is the first step of this process. By selecting the grammar the user defines the type of construct that is possible, and by selecting the library they define the set of parts available to complete the design. Determining the high level structure of a functional genetic system *de novo* could potentially be confusing to users that do not have an intimate knowledge of the role of each part in the regulation of gene expression. However, each grammar behind GenoCAD provides the design strategy for specific types of constructs. GenoCAD's default grammar, '*E. coli*-Expression Grammar', is suitable for the design of prokaryotic expression constructs. Additional grammars will be added for other applications.

The toggle switch construct was designed in nine steps as shown in Figure 2. The history pane (left side) allows users to review their work at any stage of the design process by clicking on that step. Users may click in reverse or in forward steps, and they are able to redesign from any point if they wish. Figure 3 shows a slightly condensed version of the design process of the toggle switch construct. Displayed below each part of a design are the options available to the user to transform the design. Choices in gray correspond to transformations that affect the design structure while options in white correspond to the selection of a specific part. Structural transformations are either part categories for which a specific part selection can be made or a higher level feature that must be decomposed to features lower in the abstraction hierarchy before the design can be finalized. An example of a high level part is the cistron (CIS in Figure 3), which is transformed into a RBS and gene (GEN). For each part category users have a choice of one or more specific sequences such as a specific RBS or gene. A mouse-over feature in the interface provides more information about the available choices. For example, the name of choice 04 for the promoter (PRO) category is displayed through this mechanism.

A design is complete when specific sequences have been selected for each of its structural features. It is then possible to click on the 'Download Sequence' button to export the construct sequence as a text file that can be imported into software to design oligos for gene synthesis (31–34). Alternatively the sequence synthesis can be ordered from a fast growing number of vendors providing contract gene synthesis services (1).

CUSTOM USER WORKSPACE

Parts available to all users in the public parts library have been derived from peer-reviewed publications and the



Library

Filter by grammar: E. coli Expression Grammar

Filter by library: Public Parts Library (E. coli expression grammar)

Glossary

GEN: Gene

gen01:E1010 - View sequence
gen02:J45004 - View sequence
gen03:E0020 - View sequence
gen04:E0030 - View sequence
gen05:J36801 - View sequence
gen06:J36804 - View sequence
gen07:J31007 - View sequence
gen08:J52008 - View sequence
gen09:E0040 - View sequence
gen10:E0032 - View sequence
gen11:E0022 - View sequence
gen12:J06501 - View sequence
gen13:LacI - View sequence
gen14:GFPmut3 - View sequence
gen15:cIts - View sequence
gen16:TetR - View sequence
gen17:tgGFPmut3 - View sequence

GEN-: Gene reverse

Figure 1. The GenoCAD parts library browser. Parts are associated with individual libraries, each of which is associated with a specific grammar. Users select which parts library they view through choice of a grammar and specific library in drop down boxes on the page. The part category 'Gene' is displayed in this figure along with the icon that represents genes in the designs. By clicking on the icon, the list of genes expands, allowing the user to see the available choices in the library. Selecting the link to 'View Sequence' for any part opens a small window containing the sequence of the individual part.

documentation of commercial vectors. Registered users are able to create both their own parts library and their own parts. To create a new parts library, the user selects the 'My Libraries' link on the 'Design' or 'Parts' tab. When the user selects the link to create a new library they are presented with a user-friendly interface to do so (Figure 4). Since parts libraries are associated with specific grammars, the user must select the grammar to which the new library will be linked. Parts can then be copied from other libraries linked to the same grammar by first preloading that library at the lower left of the interface. Parts can be copied into and removed from the new library with the 'add' and 'remove' buttons. Once a user has created their own library, they are then able to create new parts and associate them with that library. In Figure 5, the part creation page allows the user to specify a grammar, select which part category it belongs to, and enter the part name, sequence and description. One or more user libraries associated with the grammar must be selected at the bottom of the page before the part can be saved into specific libraries.

Users can also save their designs in their workspace. Designs can be saved and named at any stage of the design process. The 'My Designs' page provides links to delete and clone (i.e. copy) previously saved designs. By saving a design prior to selecting parts, users can

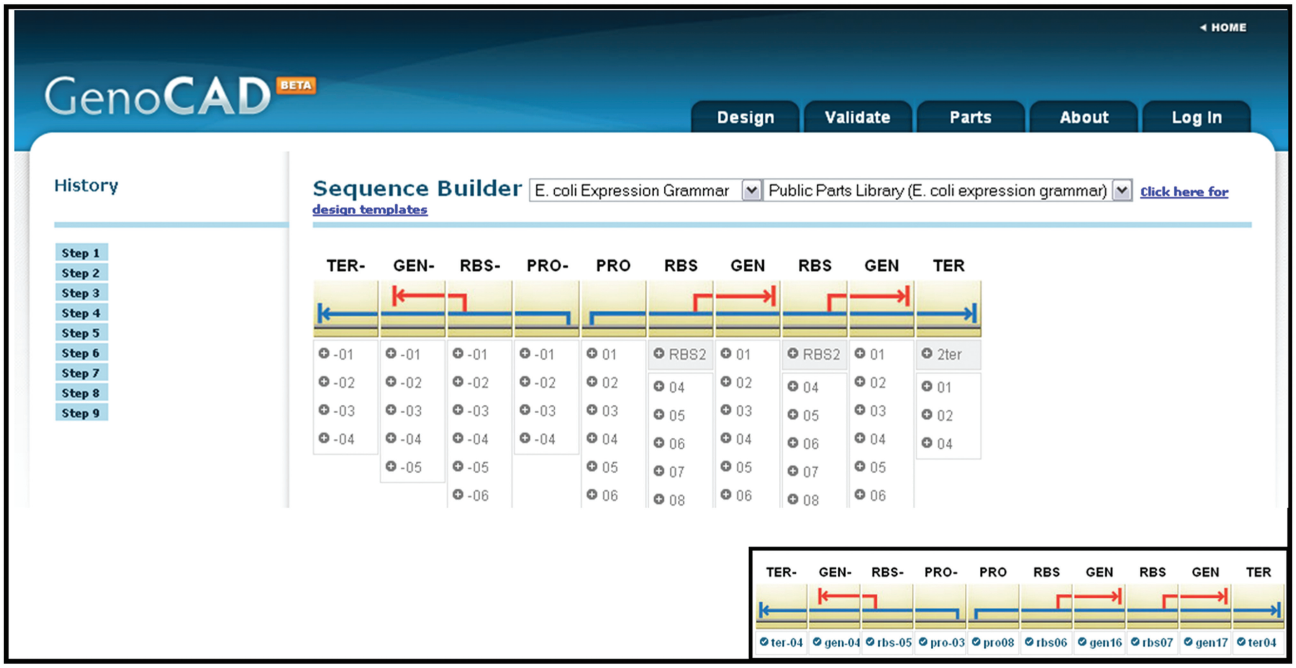
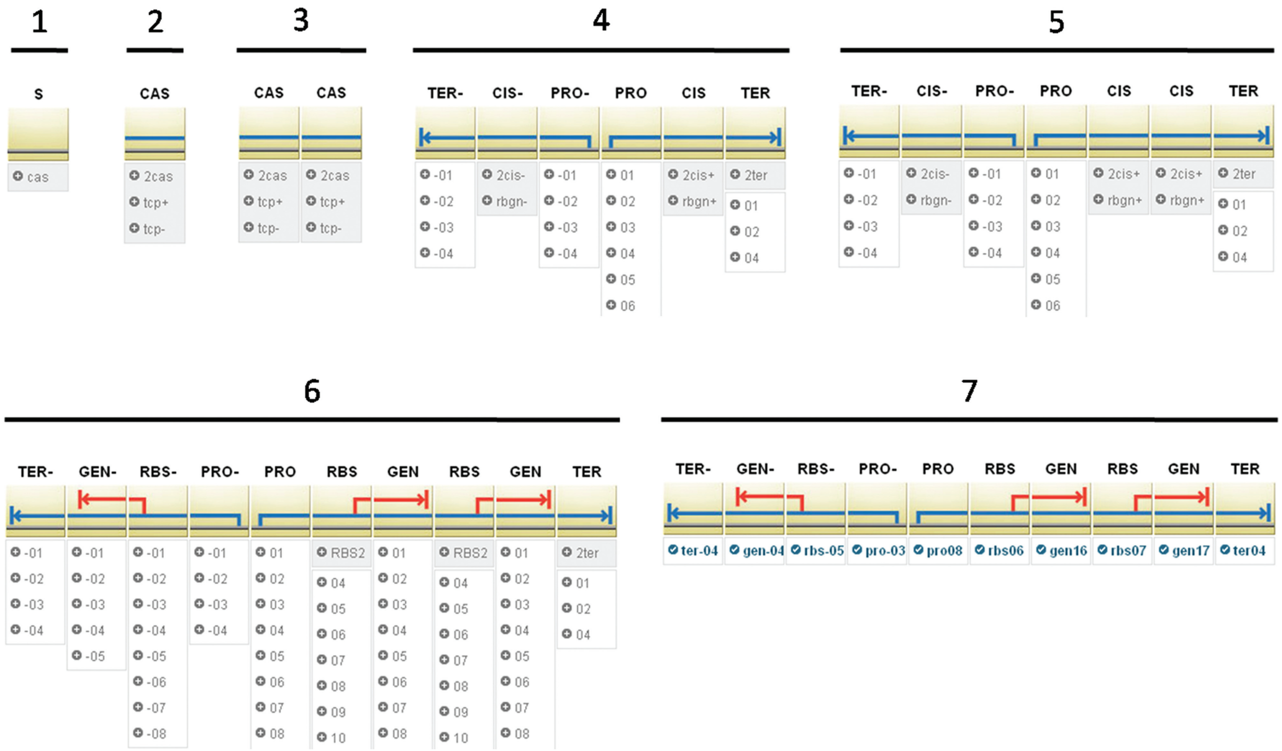

HOME
GenoCAD BETA
Design
Validate
Parts
About
Log In
History
Step 1
Step 2
Step 3
Step 4
Step 5
Step 6
Step 7
Step 8
Step 9
Sequence Builder
E. coli Expression Grammar
Public Parts Library (E. coli expression grammar)
Click here for design templates
TER- GEN- RBS- PRO- PRO RBS GEN RBS GEN TER
-01 -02 -03 -04 -05 -06
RBS2
2ter
01 02 03 04 05 06 07 08
ter-04 gen-04 rbs-05 pro-03 pro08 rbs06 gen16 rbs07 gen17 ter04

Figure 2. The design interface showing the structure of a genetic toggle switch. The interface has drop down boxes at the top to select the grammar and parts library that will be used in the design. The history panel allows users to select one of the steps in the design process and see the structure of the design at that step. Users are permitted to go back to any step and redesign from that point. The design is presented in the main panel of the page, and icons for each part and the abbreviated parts categories are shown at the top of the design. Choices for each part are shown underneath the part icon. The inset shows the final design for this construct after specific choices (terminals) are selected for each part category.


1
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CAS
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tcp+
tcp-
TER- CIS- PRO- PRO CIS TER
2cis-
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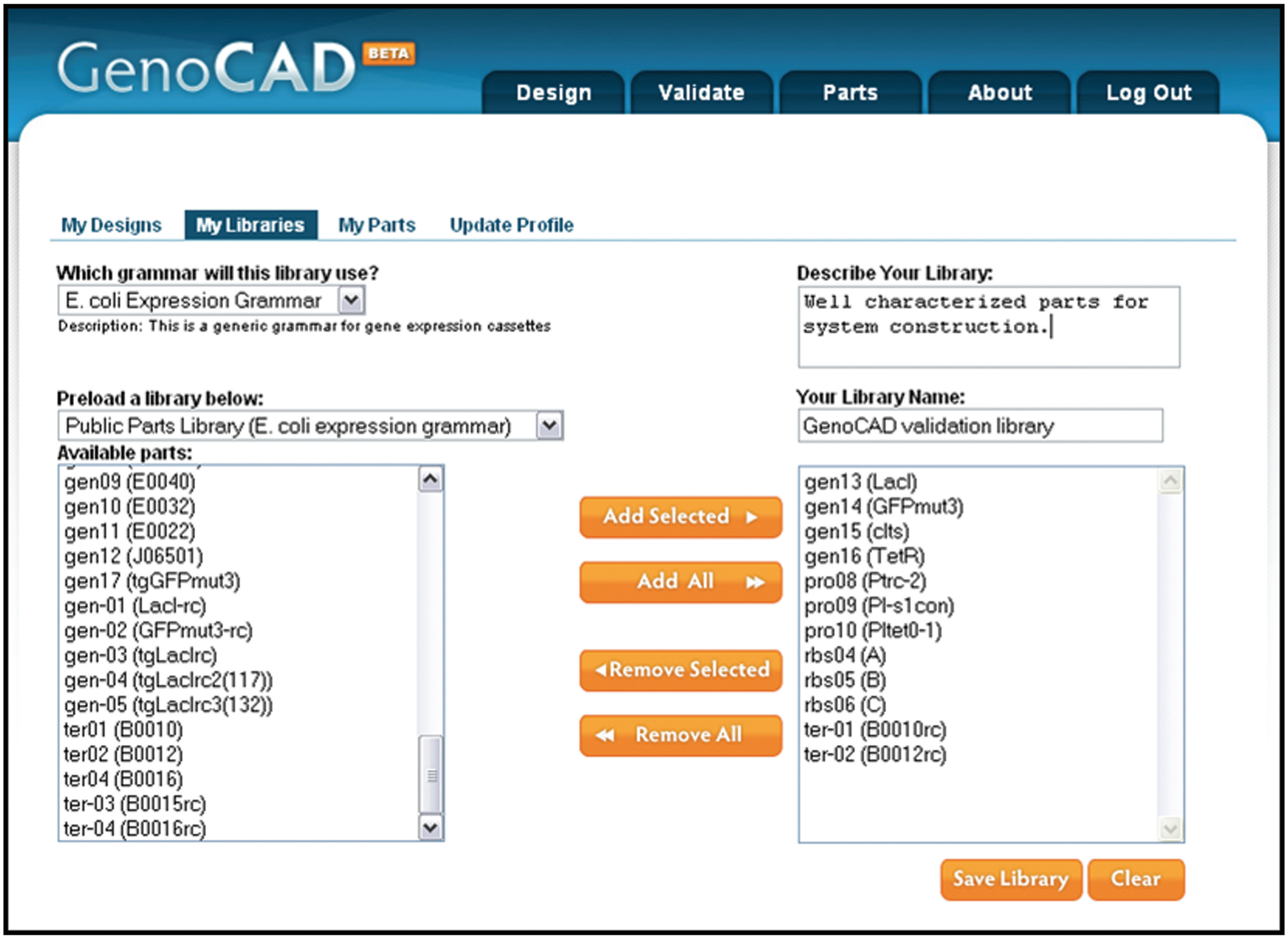
2ter
TER- GEN- RBS- PRO- PRO RBS GEN RBS GEN TER
RBS2
ter-04 gen-04 rbs-05 pro-03 pro08 rbs06 gen16 rbs07 gen17 ter04

Figure 3. A progression through the design of a bistable toggle switch. The starting symbol, S, (1) is where each design begins, and it is transformed into a transcription cassette, CAS, (2). Since the toggle switch contains two transcription cassettes, the single cassette is doubled (3). The design we are following has the cassettes oriented in opposite directions, and we achieve this by transforming the left cassette to the tpc- option, and the right to the tcp+ option (4), which contain a promoter, cistron, and terminator, but in opposite orientations. The right cassette is meant to express a transcriptional repressor and reporter gene in a bicistronic manner, so the cistron is doubled by selecting the 2cis+ option (5). Each cistron is then decomposed to a RBS and gene (6), with the RBS and gene in the reverse orientation in the left cassette. Selection of the specific promoters, RBSs, genes, and terminators produces a final construct that is associated with a DNA sequence (7).



Which grammar will this library use?
E. coli Expression Grammar
Description: This is a generic grammar for gene expression cassettes

Describe Your Library:
Well characterized parts for system construction.

Preload a library below:
Public Parts Library (E. coli expression grammar)

Your Library Name:
GenoCAD validation library

Available parts:
gen09 (E0040)
gen10 (E0032)
gen11 (E0022)
gen12 (J06501)
gen17 (tgGFPmut3)
gen-01 (LacI-rc)
gen-02 (GFPmut3-rc)
gen-03 (tgLacIrc)
gen-04 (tgLacIrc2(117))
gen-05 (tgLacIrc3(132))
ter01 (B0010)
ter02 (B0012)
ter04 (B0016)
ter-03 (B0015rc)
ter-04 (B0016rc)

gen13 (LacI)
gen14 (GFPmut3)
gen15 (cIts)
gen16 (TetR)
pro08 (Ptrc-2)
pro09 (PI-s1con)
pro10 (PltetO-1)
rbs04 (A)
rbs05 (B)
rbs06 (C)
ter-01 (B0010rc)
ter-02 (B0012rc)

Figure 4. Creating a custom parts library. Users who create an account at the website are able to create their own parts libraries, and are then able to add custom parts to these libraries. Through the library creation interface, users select the grammar that their library will belong to, provide a name for their library, and can enter a description. Parts can be added to a new library from other libraries of the same grammar, which are loaded in the lower left box on the web page. All parts or a select subset of parts, from the existing library can be copied into or removed from the new library using the orange 'add' and 'remove' buttons.

quickly clone a design template into multiple variants without having to go through the entire design process for each of them.

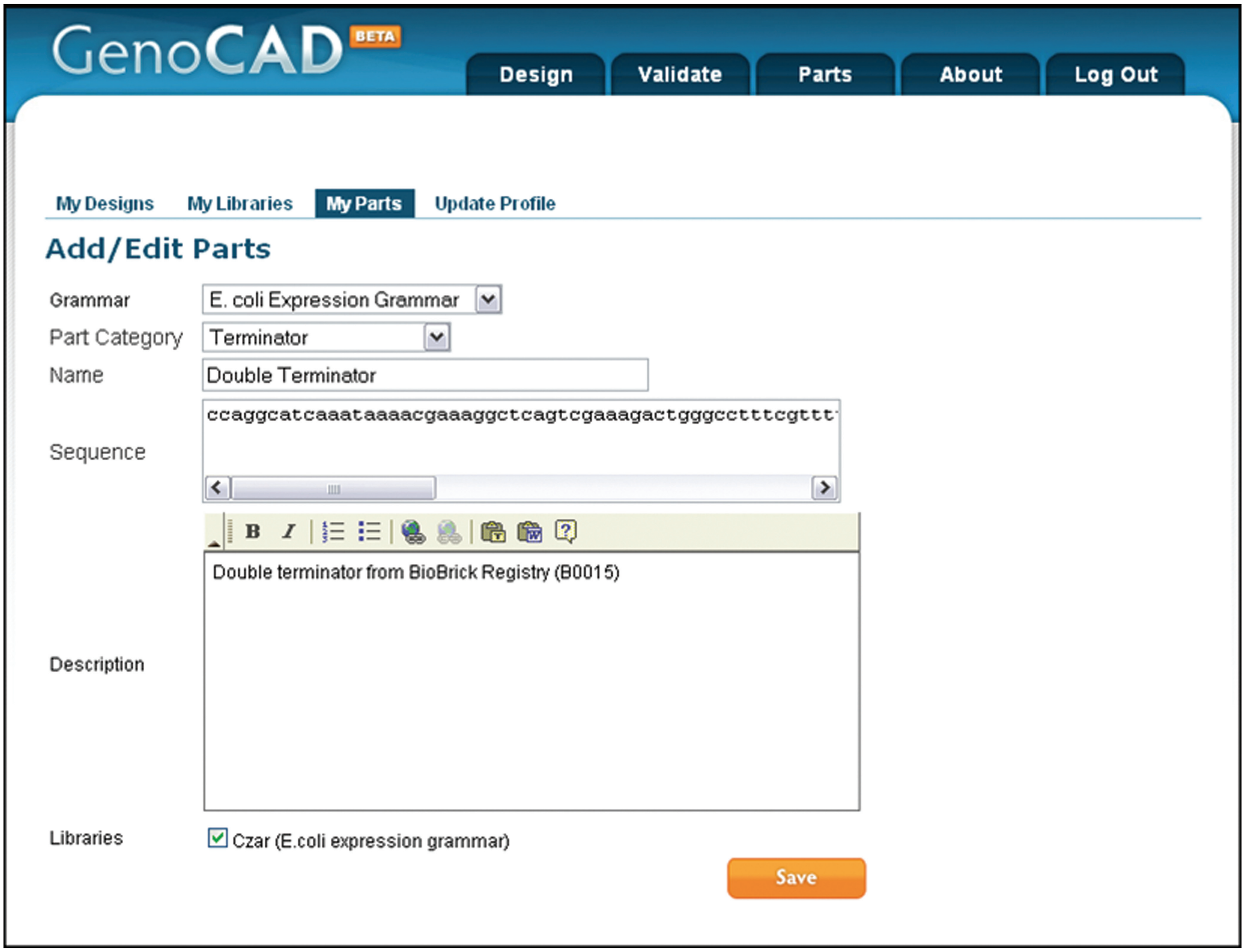
IMPLEMENTATION AND DATA MODEL

The GenoCAD website is written in a combination of PHP and JavaScript and runs on an Apache server. The MySQL database is on a different server, and both servers use the Linux operating system. The validation page relies on a custom parser developed in C++.

The data model for GenoCAD is summarized on Figure 6. Each design is associated with a specific parts library which, in turn, is linked to a specific grammar. Multiple public and user-defined libraries can be associated with each grammar and multiple designs can be associated with a specific library. Parts defined by users need to be associated with one of the user's parts libraries.

This simple data model has several limitations. Since many parts such as coding sequences can be used in different organisms, it would be desirable to replace the current hierarchical data model with a more refined model allowing the same part to be used in multiple libraries and grammars. It would also be desirable to define parts corresponding to coding regions by their amino-acid sequences instead of being limited to a DNA sequence with codons that are optimal for expression in a specific organism.

Additional grammars will be defined for the use of specific applications or for applications relevant to specific organisms in collaboration with organism or domain experts. Defining parts categories and design strategies suitable for a particular application will require a dialogue between biologists and computer scientists having experience in grammar development. Once agreed upon, grammars can easily be recorded in the MySQL database. Even though it would be attractive to guide the user in the definition of new grammars, the complexity of this process makes it unlikely that it will be possible to develop a grammar-building wizard in the foreseeable future.



Add/Edit Parts

Grammar: E. coli Expression Grammar

Part Category: Terminator

Name: Double Terminator

Sequence: ccaggcatcaaataaaacgaaaggctcagtcgaaagactgggcctttcgttt

Description: Double terminator from BioBrick Registry (B0015)

Libraries: ☑ Czar (E.coli expression grammar)

Figure 5. Interface to add a new part. Users that have created a custom library are able to add and save parts that can be used in their designs. The categories of parts permissible in designs are defined in the grammar, so the grammar must be chosen first through a drop down menu. A second drop down, then allows users to choose which part category the new part will belong to; in this case a new terminator is being created. The sequence and description of the part are entered in text boxes, and the library(s) to which the part will be added must be checked.

SUMMARY AND FUTURE WORK

Like the stand-alone Gene Designer (34) or the web-based Registry of Standard Biological Parts, GenoCAD allows users to quickly design new genetic constructs by combining sequences corresponding to various functional elements known as parts. Unlike its predecessors though, GenoCAD guides the user through a design workflow corresponding to previously agreed upon design principles captured in grammars. Since it relies on linguistic models of DNA sequences (35), GenoCAD is a tool to help users write in the language of DNA sequences.

GenoCAD is a work in progress. The sequence validation tool makes it possible to test whether a sequence developed outside of GenoCAD is consistent with a specific grammar and parts library. This tool is still rudimentary since it simply provides pass/fail information. Eventually, more sophisticated error messages will be generated to help user troubleshoot their sequence. As the GenoCAD user base grows, GenoCAD will support workgroups by allowing them to share parts, libraries and grammars.

The next major improvement to GenoCAD will include tools to predict the design behavior. By augmenting GenoCAD data model it is possible to compile a design DNA sequence into a SBML file (36) that can be simulated by one of the numerous applications that supports this standard (37). GenoCAD will then join a growing number of applications experimenting with mechanisms to derive the gene network model encoded in genetic constructs composed of standard biological parts (2,28,38,39). GenoCAD will also integrate tools to track the synthesis and assembly of designs generated in GenoCAD. Optimizing the DNA fabrication process based on the strategies used to design a series of constructs would be extremely valuable.

While the GenoCAD web site is stable and has been in operation since 2007, the experimental validation of the concepts upon which it has been developed is still ongoing.

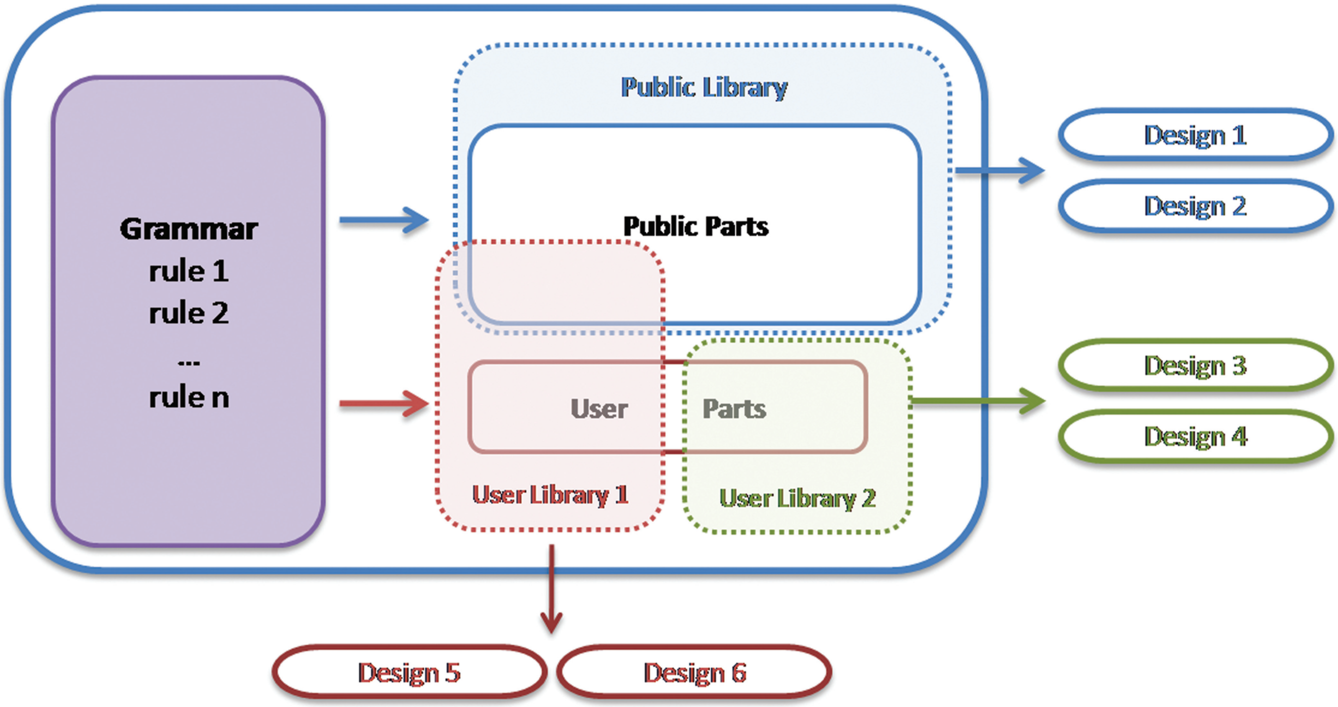


Figure 6. GenoCAD data model. Each grammar encompasses a set of rules by which constructs can be designed. The grammar also defines the categories of parts that are available to design the constructs. For each grammar there is a collection of public parts (solid, blue rectangle), which constitute a publicly available parts library (dashed, blue rectangle). 'User Libraries' can be created from any subset of the public parts, and this library can be supplemented with user-created parts (solid, red rectangle). Two user libraries (dashed, red and dashed, green rectangles) are shown here that contain different subsets of public and user-created parts. User library 2 contains all user-created parts. When a design is created, all the parts to complete the design must be contained within a single library.

As a reminder of the necessity to test designs in the lab, we will consider GenoCAD in beta test until extensive characterization of GenoCAD-designed systems has been described in peer-reviewed publications.

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REFERENCES

1. Czar,M.J., Anderson,J.C., Bader,J.S. and Peccoud,J. (2009) Gene synthesis demystified. *Trends Biotechnol.*, **27**, 63–72.
2. Goler,J.A., Bramlett,B.W. and Peccoud,J. (2008) Genetic design: rising above the sequence. *Trends Biotechnol.*, **26**, 538–544.
3. Graslund,S., Nordlund,P., Weigelt,J., Hallberg,B.M., Bray,J., Gileadi,O., Knapp,S., Oppermann,U., Arrowsmith,C., Hui,R. *et al.* (2008) Protein production and purification. *Nat. Methods*, **5**, 135–146.
4. Ghaemmaghami,S., Huh,W.K., Bower,K., Howson,R.W., Belle,A., Dephoure,N., O'Shea,E.K. and Weissman,J.S. (2003) Global analysis of protein expression in yeast. *Nature*, **425**, 737–741.
5. Huh,W.K., Falvo,J.V., Gerke,L.C., Carroll,A.S., Howson,R.W., Weissman,J.S. and O'Shea,E.K. (2003) Global analysis of protein localization in budding yeast. *Nature*, **425**, 686–691.
6. Keasling,J.D. (2008) Synthetic biology for synthetic chemistry. *ACS Chem. Biol.*, **3**, 64–76.
7. Keasling,J.D. and Chou,H. (2008) Metabolic engineering delivers next-generation biofuels. *Nat. Biotechnol.*, **26**, 298–299.
8. Cai,L., Dalal,C.K. and Elowitz,M.B. (2008) Frequency-modulated nuclear localization bursts coordinate gene regulation. *Nature*, **455**, 485–490.
9. Guido,N.J., Wang,X., Adalsteinsson,D., McMillen,D., Hasty,J., Cantor,C.R., Elston,T.C. and Collins,J.J. (2006) A bottom-up approach to gene regulation. *Nature*, **439**, 856–860.
10. Murphy,K.F., Balazsi,G. and Collins,J.J. (2007) Combinatorial promoter design for engineering noisy gene expression. *Proc. Natl Acad. Sci. USA*, **104**, 12726–12731.
11. Cox,R.S. 3rd, Surette,M.G. and Elowitz,M.B. (2007) Programming gene expression with combinatorial promoters. *Mol. Syst. Biol.*, **3**, 145.
12. Cello,J., Paul,A.V. and Wimmer,E. (2002) Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. *Science*, **297**, 1016–1018.
13. Chan,L.Y., Kosuri,S. and Endy,D. (2005) Refactoring bacteriophage T7. *Mol. Syst. Biol.*, **1**, 2005 0018.
14. Coleman,J.R., Papamichail,D., Skiena,S., Futcher,B., Wimmer,E. and Mueller,S. (2008) Virus attenuation by genome-scale changes in codon pair bias. *Science*, **320**, 1784–1787.
15. Tumpey,T.M., Basler,C.F., Aguilar,P.V., Zeng,H., Solorzano,A., Swayne,D.E., Cox,N.J., Katz,J.M., Taubenberger,J.K., Palese,P. *et al.* (2005) Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science*, **310**, 77–80.
16. Gibson,D.G., Benders,G.A., Andrews-Pfannkoch,C., Denisova,E.A., Baden-Tillson,H., Zaveri,J., Stockwell,T.B., Brownley,A., Thomas,D.W., Algire,M.A. *et al.* (2008) Complete chemical synthesis, assembly, and cloning of a Mycoplasma genitalium genome. *Science*, **319**, 1215–1220.
17. Dymond,J.S., Scheifele,L.Z., Richardson,S., Lee,P., Chandrasegaran,S., Bader,J.S. and Boeke,J.D. (2009) Teaching

synthetic biology, bioinformatics and engineering to undergraduates: the interdisciplinary build-a-genome course. *Genetics*, **181**, 13–21.

18. Bains,W. (2001) The parts list of life. *Nat. Biotechnol.*, **19**, 401–402.
19. Baker,D., Church,G., Collins,J., Endy,D., Jacobson,J., Keasling,J., Modrich,P., Smolke,C. and Weiss,R. (2006) Engineering life: building a fab for biology. *Sci. Am.*, **294**, 44–51.
20. Endy,D. (2005) Foundations for engineering biology. *Nature*, **438**, 449–453.
21. Isaacs,F.J., Dwyer,D.J., Ding,C., Pervouchine,D.D., Cantor,C.R. and Collins,J.J. (2004) Engineered riboregulators enable post-transcriptional control of gene expression. *Nat. Biotechnol.*, **22**, 841–847.
22. Gardner,T.S., Cantor,C.R. and Collins,J.J. (2000) Construction of a genetic toggle switch in Escherichia coli. *Nature*, **403**, 339–342.
23. Atkinson,M.R., Savageau,M.A., Myers,J.T. and Ninfa,A.J. (2003) Development of genetic circuitry exhibiting toggle switch or oscillatory behavior in Escherichia coli. *Cell*, **113**, 597–607.
24. Elowitz,M.B. and Leibler,S. (2000) A synthetic oscillatory network of transcriptional regulators. *Nature*, **403**, 335–338.
25. Stricker,J., Cookson,S., Bennett,M.R., Mather,W.H., Tsimring,L.S. and Hasty,J. (2008) A fast, robust and tunable synthetic gene oscillator. *Nature*, **456**, 516–519.
26. Ball,P. (2007) Synthetic biology: designs for life. *Nature*.
27. Drubin,D.A., Way,J.C. and Silver,P.A. (2007) Designing biological systems. *Genes Dev.*, **21**, 242–254.
28. Marchisio,M.A. and Stelling,J. (2008) Computational design of synthetic gene circuits with composable parts. *Bioinformatics*, **24**, 1903–1910.
29. Peccoud,J., Blauvelt,M.F., Cai,Y., Cooper,K.L., Crasta,O., DeLalla,E.C., Evans,C., Folkerts,O., Lyons,B.M., Mane,S.P. *et al.* (2008) Targeted Development of Registries of Biological Parts. *PLoS ONE*, **3**, e2671.
30. Cai,Y., Hartnett,B., Gustafsson,C. and Peccoud,J. (2007) A syntactic model to design and verify synthetic genetic constructs derived from standard biological parts. *Bioinformatics*, **23**, 2760–2767.
31. Hoover,D.M. and Lubkowski,J. (2002) DNAWorks: an automated method for designing oligonucleotides for PCR-based gene synthesis. *Nucleic Acids Res.*, **30**, e43.
32. Jayaraj,S., Reid,R. and Santi,D.V. (2005) GeMS: an advanced software package for designing synthetic genes. *Nucleic Acids Res.*, **33**, 3011–3016.
33. Richardson,S.M., Wheelan,S.J., Yarrington,R.M. and Boeke,J.D. (2006) GeneDesign: rapid, automated design of multikilobase synthetic genes. *Genome Res.*, **16**, 550–556.
34. Villalobos,A., Ness,J.E., Gustafsson,C., Minshull,J. and Govindarajan,S. (2006) Gene Designer: a synthetic biology tool for constructing artificial DNA segments. *BMC Bioinformatics*, **7**, 285.
35. Searls,D.B. (2002) The language of genes. *Nature*, **420**, 211–217.
36. Hucka,M., Finney,A., Sauro,H.M., Bolouri,H., Doyle,J.C., Kitano,H., Arkin,A.P., Bornstein,B.J., Bray,D., Cornish-Bowden,A. *et al.* (2003) The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics*, **19**, 524–531.
37. Hoops,S., Sahle,S., Gauges,R., Lee,C., Pahle,J., Simus,N., Singhal,M., Xu,L., Mendes,P. and Kummer,U. (2006) COPASI – a COmplex PAthway SImulator. *Bioinformatics*, **22**, 3067–3074.
38. Hill,A.D., Tomshine,J.R., Weeding,E.M., Sotiropoulos,V. and Kaznessis,Y.N. (2008) SynBioSS: the synthetic biology modeling suite. *Bioinformatics*, **24**, 2551–2553.
39. Rodrigo,G., Carrera,J. and Jaramillo,A. (2007) Asmparts: assembly of biological model parts. *Syst. Synth. Biol.*, **1**, 167–170.